(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 March 2002 (14.03.2002)

PCT

(10) International Publication Number
WO 02/020022 A1

(51) International Patent Classification[7]: A61K 31/65

(21) International Application Number: PCT/US01/27593

(22) International Filing Date:
6 September 2001 (06.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/230,350 6 September 2000 (06.09.2000) US

(71) Applicant *(for all designated States except US)*: NEUROTHERAPEUTICS, LLC [US/US]; 137 Nautilus Drive, Madiosn, WI 53705 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: DUNCAN, Ian, D. [US/US]; 137 Nautilus Drive, Madison, WI 53705 (US).

(74) Agents: WELCH, Teresa, J. et al.; Michael Best & Friedrich LLP, Suite 700, One South Pinckney Street, P.O. Box 1806, Madison, WI 53701-1806 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declaration under Rule 4.17:**
— *of inventorship (Rule 4.17(iv)) for US only*

**Published:**
— *with international search report*
— *with amended claims and statement*

**Date of publication of the amended claims and statement:**
11 July 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 02/020022 A1

(54) Title: METHOD OF TREATING NEUROLOGIC DISORDERS

(57) Abstract: The invention provides a method for treating certain neurological diseases by administering to a patient in need thereof an effective amount of tetracycline compound.

**AMENDED CLAIMS**

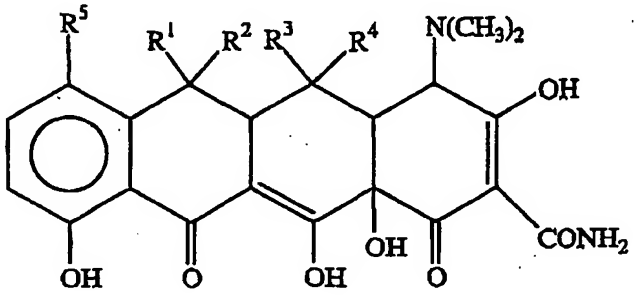
[received by the International Bureau on 1 March 2002 (01.03.02);
original claim 1 amended; new claims 22-31 added;
remaining claims unchanged (3 pages)]

1. A method of treating or preventing a disease in a mammal comprising administering an effective amount of a tetracycline compound, the disease being Alzheimers' disease, Guillain Barré syndrome, adreneoleukodystrophy, Parkinson's disease, or amyotrophic lateral sclerosis.

2. A method of downregulating microglia expression in a mammal, comprising administering to the mammal in need thereof an effective amount of a tetracycline compound.

3. A method of inhibiting inflammatory activity associated with microglial activation and production, comprising administering to a mammal in need thereof an effective amount of a tetracycline compound.

4. The method of claim 1 wherein the tetracycline compound is a compound of formula (I)




(I)

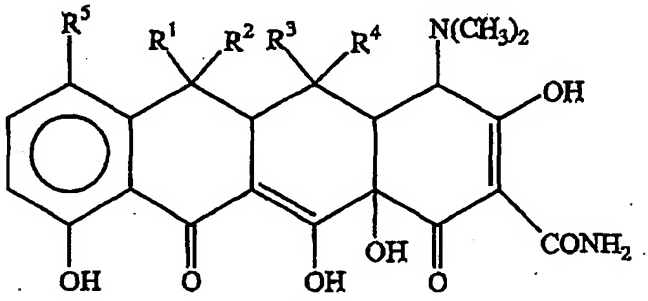
wherein $R^1$ is $CH_3$ or OH and $R^2$ is H or OH, or $R^1$ and $R^2$ taken together are $=CH_2$, $R^3$ and $R^4$ are H or OH and $R^5$ is Cl or $N(CH_3)_2$.

5. The method of claim 2 wherein the tetracycline compound is a compound of formula (I)

20. The method of claim 1 wherein the tetracycline compound is administered parenterally, internally or via a controlled release formulation.

21. The method of claim 1 wherein the amount is below the antibiotic effective amount.

22. A method of treating or preventing a neurologic disease in a mammal comprising administering to the mammal suffering therefrom an effective amount of a tetracycline compound of formula (I)




(I)

wherein $R^1$ is $CH_3$ or OH and $R^2$ is H or OH, or $R^1$ and $R^2$ taken together are $=CH_2$, $R^3$ and $R^4$ are H or OH and $R^5$ is Cl or $N(CH_3)_2$, the disease being Alzheimers' disease, Guillain Barré syndrome, adreneoleukodystrophy, Parkinson's disease, or amyotrophic lateral sclerosis.

23. The method of claim 22 wherein the disease is Alzheimers' disease,

24. The method of claim 22 wherein the disease is Guillain Barré syndrome.

25. The method of claim 22 wherein the disease is adreneoleukodystrophy.

26. The method of claim 22 wherein the disease is Parkinson's disease.

27. The method of claim 22 wherein the disease is amyotrophic lateral sclerosis.

28. The method of claim 22 wherein the amount about 0.1 mg/kg/day to about 45 mg/kg/day.

29. The method of claim 28 wherein the amount is about 0.1 mg/kg/day to about 18 mg/kg/day.

30. The method of claim 22 wherein the amount is sufficient to reduce symptoms of the disease at least 50% compared to pre-treatment symptoms.

31. The method of claim 22 wherein the administering is done for a period of about 2 to 3 weeks or until the mammal becomes asymptomatic of the disease.

## Statement under Article 19(1)

In the International Search Report, a single reference, Yrjanheikki et al., Abstract Database Medline, *Proc. Nat. Acad. Sci. (1998) 95(26) 15769-74*, has been cited as novelty-destroying as to claims 2, 3, 5, 6, 8, 10-13, 15 and 16, and rendering claims 1, 4, 7, 9, 14, and 17-21 as lacking inventive step. The reference is understood to be cited as teaching that certain compounds of the tetracycline class are useful in the treatment of brain ischemia.

The gravamen of the Report is understood to be that utility of tetracycline compounds for brain ischemia can be extropolated to long-term neurodegenerative diseases of the brain, treatment of which is the subject matter of the claimed invention. Applicant respectfully traverses such conclusion.

The time of onset between brain ischemia and the neurologic diseases addressed by the present invention is strikingly disparate. Brain ischemia is an acute event. The condition is associated with an inadequate flow of oxygenated blood to a part of the brain, caused by the constriction or blockage of the blood vessels supplying it. Ischemia occurs any time that blood flow to a tissue is reduced below a critical level. This reduction in blood flow can result from the blockage or breakage of a vessel.

When an ischemic event occurs, there is a gradation of injury that arises from the ischemic site. The cells at the site of blood flow restriction, undergo necrosis and form the core of a lesion. A penumbra is formed around the core where the injury is not immediately fatal but progresses slowly toward cell death. This progression to cell death may be reversed upon reestablishment of blood flow within a short time of the ischemic event.

In contrast, the neurodegenerative diseases such as Alzheimer's are characterized by progressive and irreversible loss of neurons from specific regions of the brain. They are primarily disorders of later life, developing in individuals who are neurologically normal. Applicant has found tetracycline compounds to be of value in treating these diseases. While there may be some suggested commonality of the presence of one cell type in brain ischemia and neural degeneration, this amounts to no more than a invitation to experiment. Such invitation to experiment or "obvious to try" is not the measure by which a claim is determined to be lacking in inventive step. As such, the skilled person with the teachings of the Yrjanheikki et al., Abstract in hand would not have been guided to the claimed invention, and would not have had a reasonable expectation of success in treating neurologic diseases that have long been treatment-resistant.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 March 2002 (14.03.2002)

PCT

(10) International Publication Number
WO 02/20022 A1

(51) International Patent Classification[7]: A61K 31/65

(21) International Application Number: PCT/US01/27593

(22) International Filing Date:
6 September 2001 (06.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/230,350 6 September 2000 (06.09.2000) US

(71) Applicant *(for all designated States except US)*: **NEUROTHERAPEUTICS, LLC** [US/US]; 137 Nautilus Drive, Madiosn, WI 53705 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **DUNCAN, Ian, D.** [US/US]; 137 Nautilus Drive, Madison, WI 53705 (US).

(74) Agents: **WELCH, Teresa, J.** et al.; Michael Best & Friedrich LLP, Suite 700, One South Pinckney Street, P.O. Box 1806, Madison, WI 53701-1806 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declaration under Rule 4.17:**
— *of inventorship (Rule 4.17(iv)) for US only*

**Published:**
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 02/20022 A1

(54) Title: METHOD OF TREATING NEUROLOGIC DISORDERS

(57) Abstract: The invention provides a method for treating certain neurological diseases by administering to a patient in need thereof an effective amount of tetracycline compound.

# METHOD OF TREATING NEUROLOGIC DISORDERS

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority from U.S. Provisional Application 60/230,350, filed 6 September 2000, which is incorporated herein by reference.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not Applicable

This invention relates to a method of treating neurologic disorders including Alzheimer's disease, Guillain Barré Syndrome, adreneoleukodystrophy, Parkinson's disease, and amyotrophic lateral sclerosis, by administering an effective amount of a tetracycline compound.

Alzheimer's disease is probably the best known central nervous system (CNS) disorder. It is the most prevalent human neurodegenerative disease and creates untold human suffering at a huge social and economic cost worldwide. The cause of the disease is unknown and there are no cures nor, indeed, treatments that in any way ameliorate the disease course. Affected patients develop classic brain changes with the formation of amyloid plaques, neurofibrillary tangles, inflammation and degeneration of neurons. As a result of these changes, patients develop progressive memory loss that leads to dementia and total reliance on others.

While there have been recent advances in experimental therapies for Alzheimer's disease, none is close to being used in human treatment. Thus, there is a critical need for the discovery of new drugs or new applications of existing drugs to treat devastating, chronic neurologic disorders such as Alzheimer's disease.

One proposed therapeutic approach is to minimize or prevent the inflammatory changes that occur in the brains of, e.g., Alzheimer patients (Selkoe 1999). It is thought that a key cell in the cellular cascade that results in neuronal death in Alzheimer's disease is the microglial cell. These cells are associated with amyloid plaques and produce a

number of pro-inflammatory cytokines that can result in neuronal death. Thus, use of anti-inflammatory agents may be of value in the treatment of neurologic disorders.

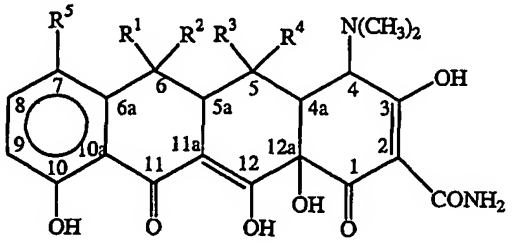
Second generation tetracycline drugs, well-known for their antibiotic effects, also have known anti-inflammatory effects and could have therapeutic use in many serious central nervous system (CNS) disorders. It has been reported that minocycline, a tetracycline compound, reduced the microglial response in the area surrounding areas of brain ischemia in an experimental model of stroke, and that it protects neurons against death in tissue culture (Yrjänheikki *et al.* 1999, 1998).

The present invention provides a method of treating neurologic disorders which includes administering an effective amount of a tetracycline compound of formula (I), described hereinafter. In another aspect, the invention provides a method of providing to a mammalian system an effective amount of a tetracylcine compound sufficient to down regulate microglia expression, activation and production, and hence, prevent, reduce or minimize inflammation.

These and other advantages and a fuller appreciation of the specific attributes of this invention will be gained upon an examination of the detailed description of preferred embodiments and appended claims.

This invention relates to a method of treating neurologic disorders such as Alzheimer's disease, Guillain Barré Syndrome, adreneoleukodystrophy, Parkinson's disease, and amyotrophic lateral sclerosis, by administering an effective amount of a tetracycline compound. The present invention is directed to the prevention or treatment of a broad spectrum of diseases, which may be linked to microglial activation and inflammation. The method can be used to prevent, inhibit or alleviate any condition in which results from upregulation of expression or activity of microglia.

In one aspect, a tetracycline compound suitably in accordance with the present invention is set forth in formula (I):




(I)

wherein $R^1$ is $CH_3$ or OH and $R^2$ is H or OH, or $R^1$ and $R^2$ taken together are $=CH_2$, $R^3$ and $R^4$ are H or OH and $R^5$ is Cl or $N(CH_3)_2$. Compounds of formula (I) of particular value in accordance with the present invention are minocycline and doxycycline, i.e., wherein $R^1$, $R^2$, $R^3$ and $R^4$ are H and $R^5$ is $N(CH_3)_2$; and $R^1$ is $CH_3$, $R^2$ is H, $R^3$ is OH, and $R^4$ and $R^5$ are H, respectively. Compounds of formula (I) generally have antibiotic activity.

However, non-antibiotic tetracyclines, i.e., those that have little or no antibiotic activity, are also contemplated within the scope of the method in accordance with the present invention. Mitscher (1978) has reviewed the modifications to the basic tetracycline structure and their effect on retention of antibiotic properties. According to Mitscher, modifications at positions 5-9 of the tetracycline ring system can be made without causing the complete loss of antibiotic properties. However, changes to the basic structure of the rings system, or replacement or deletion of substituents at position 1-4 or 1-12, generally lead to synthetic tetracyclines with substantially less, or essentially no, antibacterial, antimicrobial, i.e., non-antibiotic, activity. The compounds are often referred to as chemically modified tetracyclines or CMTs. Many CMTs have been synthesized. For example, 4-dedimethylaminotetracycline, i.e., deletion of the $N(CH_3)_2$ group at the C-4 position, is commonly considered to be a non-antibacterial, non-antimicrobial tetracycline.

Other examples of such tetracyclines include 6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 4-dedimethyl-5-oxytetracycline,4-hydroxy-4-dedimethylaminotetracycline, 5α,6-anhydro-4-hydroxy-4-dedimethylaminotetracycline, 6α-deoxy-5-hydroxy-4-dedimethylaminotetracycline, 4-dedimethylaminotetracycline, 4-dedimethylamino-12α-deoxytetracycline, 6α-deoxy-5-hydroxy-4-dedimethylaminotetracycline, tetracyclinonitrile, 6α-benzylthiomethylenetetracycline, mono-N-alkylated amide of tetracycline, 6-fluoro-6-demethyltetracycline, and 11α-chlorotetracycline.

Thus, compounds of formula (I) as well as compounds in which the basic tetracycline structure has been altered to render a compound with little or no antibiotic activity are suitable in accordance with the present invention.

During recent years, it has been established that tetracyclines, which are rapidly absorbed and have a prolonged half-life, exert biological effects independent of their

antimicrobial activity (Golub et al. 1991; Golub et al. 1992; Uitto et al. 1994). Such effects include inhibition of matrix metalloproteinases, including collagenase gelatinase and stromelysin which have been implicated in rheumatoid arthritis. These metalloproteinases are known to be upregulated in osteoarthritis-affected joints (Greenwald 1994; Mohtai et al. 1993). It has also been shown that prophylactic administration of doxycycline (a semisynthetic tetracycline) markedly reduced the severity of osteoarthritis in dog models (Yu et al (1992)), and that minocycline (a semisynthetic tetracycline) is safe and effective for patients with mild and moderate arthritis (Tilley et al. 1995). More, recent studies have also suggested that tetracyclines and inhibitors of metalloproteinases inhibit tumor progression (DeClerck et al. 1994), bone resorption (Rifkin et al 1994) and angiogenesis (Maragoudakis et al. 1994) and have anti-inflammatory properties (Ramamurthy et al. 1994).

It has now been found that tetracyclines may be of value in treating certain neurologic disorders. The amount of the tetracycline compound used according to the present invention is an amount that is effectively inhibitory of microglial expression or activity. An amount of a tetracycline compound is effectively inhibitory of or downregulates microglia activity if it significantly reduces microglia expression or activity, or if it reduces microglial cell production. The amount is suitably an anti-inflammatory effective amount.

The tetracycline compounds useful in accordance with the present invention appear to exhibit their beneficial effect in a dose-dependent manner. Thus, within broad limits, administration of larger quantities of a tetracycline compound is expected to inhibit microglia activation to a greater degree than does administration of a smaller amount. Moreover, efficacy is also contemplated at dosages below the level at which toxicity is seen. Further, in practice, higher doses of the compounds of the present invention are generally used where the therapeutic treatment of a disease state is the desired end, while the lower doses are generally used for prophylactic purposes.

It will be appreciated that the specific dosage administered in any given case will be adjusted in accordance with the specific compounds being administered, the disease to be treated, the condition of the subject and other relevant medical factors that may modify the activity of the drug or the response of the subject, as is well known by those skilled in the art. For example, the specific dose for a particular patient depends on age,

body weight, general state of health, on diet, on the timing and mode of administration, on the rate of excretion, and on medicaments used in combination and the severity of the particular disorder to which the therapy is applied. Dosages for a given patient can be determined using conventional considerations, e.g., by customary comparison of the differential activities of the subject compounds and of a known agent, such as by means of an appropriate conventional pharmacological protocol.

The maximal dosage for a subject is the highest dosage, which does not cause undesirable or intolerable side effects. For example, it is contemplated that doses from about 0.1 mg/kg/day to about 45 mg/kg/day, and suitably, from about 1 mg/kg/day to about 18 mg/kg/day are generally effective. Such dosages are generally considered below the antibiotic effective dose, typically about 45mg/kg/day. Treatment would be given for about 2-3 weeks or until full recovery or until the patient becomes asymptomatic. It is anticipated that dosages of the tetracycline compound in accordance with the present invention will reduce symptoms at least 50% compared to pre-treatment symptoms.

Systemic administration of tetracycline compounds is contemplated in accordance with the present invention, especially those tetracycline compounds capable of substantial absorption and effective systemic distribution.

A preferred pharmaceutical composition for use in the method in accordance with the present invention includes a combination of the tetracycline compound in a suitable pharmaceutical carrier (vehicle) or excipient as understood by practitioners in the art. Parenteral administration (e.g., intravenous injection) is a desirable route of delivery of the tetracycline. For parenteral application, particularly suitable are injectable, sterile solutions, oily or aqueous solution, as well as suspensions, emulsions, or implants, including suppositories. Ampoules are convenient unit dosage forms. The compositions for administration may include the tetracycline compound with appropriate diluents, carriers, and the like which are readily formulated.

Enteral use is also contemplated, and formulations such as tablets, liquids, drops, lozenges or capsules, can be employed to provide the compound. A syrup, elixir or the like can be used if a sweetened vehicle is desired.

Alternatively, delivery can by sustained, delayed release (i.e., controlled release) to provide a constant serum level of the tetracycline compound. Many controlled release systems for controlling the release of an active ingredient over the course of several hours

are known, e.g., the wax matrix system, the coated granular, the "miniature osmotic pump" system and the Forest Synchron System of Forest Laboratories.

Also included within the scope of the present invention is the co-administration of the tetracycline compound in accordance with the present invention with one or more adjunct agent capable of inhibiting inflammation in tissue, e.g., steroidal and non-steroidal anti-inflammatory drugs. The term "co-administration" includes administration of two or more agents in a single unitary dosage form, administration of agents simultaneously (i.e., at the same time), and administration of agents sequentially. It is anticipated that a compound of formula (I) used in combination with various anti-inflammatory agents can give rise to a significantly enhanced anti-inflammatory effect, thus providing an increased therapeutic effect. Specifically, as a significantly increased anti-inflammatory effect is obtained with the above disclosed combinations utilizing lower concentrations of the anti-inflammatory drugs compared to treatment regimes in which the drugs are used alone, there is the potential to provide therapy wherein adverse side effects associated with anti-inflammatory agents are considerably reduced than normally observed with the anti-inflammatory agents used alone in larger doses.

The following examples are provide to assist in a further understanding of the invention. The particular materials and conditions employed are intended to be further illustrative of the invention and are not limiting upon the reasonable scope thereof.

**Example 1:** Alzheimer's disease

A first set of experiments involves a model system which is a transgenic mouse that overexpresses (i.e., produces a gene product that exceeds levels of production in normal or non-transformed organisms) a mutant form of the human amyloid-β precursor protein. Two sets of experiments are performed.

In the first, the transgenic mice are treated with a tetracycline compound, e.g., minocycline (45mg/kg/day) from the onset of pathological change for a period of 2-3 months and then the brains of the treated mice are studied morphologically. Differences evaluating inflammation, plaque formation and neuronal death are determined between this group and those receiving no treatment.

In a second set of experiments, a group of treated animals are studied for a shorter period of time as to the ability to resolve pathological changes after they have been present for some time.

Another set of experiments is performed utilizing an *in vitro* system. A protocol, established by Coombs et al (2000), is used in which microglia are added to neurons in culture and to which amyloid-β peptides are added. Minocycline is then be added to these cultures and the expression of cytokines known to kill neurons is evaluated as is the survival of these cells in the same culture system. This experimental approach allows manipulation of variables in the system (i.e. dosage, other drugs) and the data generated is applicable to *in vivo* experimental models and, of course, therapeutic trials in humans.

**Example 2:** Guillain Barré Syndrome (GBS):

Guillain Barré Syndrome is a neuropathy that shares many pathologic hallmarks with experimental allergic encephalomyelitis (EAE) except that inflammation, demyelination and degeneration are limited to the peripheral nervous system (PNS). Like EAE , an animal model exists which is called experimental allergic neuritis (EAN). It has been reported that, in EAE, minocycline prevents the invasion of inflammatory cells into the CNS. The goal in EAN will be to stop macrophages from infiltrating into the PNS. Such an approach is contemplated to have important therapeutic applications in GBS (Griffin et 1990).

Two sets of experiments are performed. In the first, rats, immunized with peripheral myelin in Freund's adjuvant, are be treated 3-5 days prior to onset of clinical signs and every day thereafter. A control group is untreated. The rats are monitored for the development of clinical signs and the severity scored. Two to three weeks after this, they are perfused and the PNS studied morphologically.

In the second experiment, a tetracycline compound, e.g., minocycline, is given at the onset of clinical signs, and the animals observed thereafter to determine the prevention of progression of the disease and a shortening of the clinical course.

**Example 3:** Other neurologic diseases

Inflammation and microglial activation have also been noted to be a significant component of the neuropathology of Parkinson's disease, amyotrophic lateral sclerosis —

Lou Gehrig's disease, adrenoleukodystrophy and AIDS encephalopathy. In addition, there has been a recent suggestion that microglia may play a key role in the development of brain abnormalities in patients with schizophrenia (Munn 2000). Based on the data presented above, there seems to be sufficient rationale to consider the compounds of the present invention as a therapy in each of these disorders.

In summary, the present invention provides a method of treating neurologic disorders by administering an effective amount of a tetracycline compound. While not wanting to be bound by any particular theory, it is thought that the anti-inflammatory activity of the compounds of the present invention provides a basis for the compounds' therapeutic value in neurologic disorder.

While the present invention has now been described and exemplified with some specificity, those skilled in the art will appreciate the various modifications, including variations, additions and omissions, that may be made in what has been described. Accordingly, it is intended that these modifications also be encompassed by the present invention and that the scope of the present invention be limited solely be the broadest interpretation that lawfully can be accorded the appended claims.

REFERENCES:

Selkoe, D.J., 1999, Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* 399:A23-A30.

Yrjänheikki J., Tikka T., Keinänen, R., Goldsteins, G., Chan, P.H., Koistinaho, J., 1999, A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc. Natl. Acad. Sci. USA* 96:13496-13500.

Yrjänheikki J., Keinänen, R., Pellikka, M., Hokfelt, T., Koistinaho, J., 1998, Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc. Natl. Acad. Sci. USA* 95:15769-15774.

Combs, C.K., Johnson, D.E., Karlo, J.C., Cannady S.B., Landreth, G.E., 2000, Inflammatory mechanisms in Alzheimer's disease: Inhibition of β-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARγ agonists. *The Journal of Neuroscience* 20: 558-567.

Griffin, J.W., Stoll, G., Li, C.Y., Tyor, W., Cornblath, D.R., 1990, Macrophage responses in inflammatory demyelinating neuropathies. *Ann. Neurol.* 27 Suppl. S64-S68.

Matsushima, G.K., Taniike, M., Glimcher, L.H., Grusby, M.J., Frelinger, J.A., Suzuki, K., Ting, J.P-Y, 1994, Absence of MHC class II molecules reduces CNS demyelination, microglial/macrophage infiltration, and twitching in murine globoid cell leukodystrophy. *Cell* 78:645-656.

Munn, N.A., 2000, Microglia dysfunction in schizophrenia: An integrative theory. *Med. Hypotheses* 54:198-202.

DeClerck, Y.A., Shimada, H., Taylor, S.M. & Langley, K.E. (1994) *Annals N.Y. Acad. Sci.* 732, 222-232.

Golub, L.M., Ramamurthy, N.S. & McNamara, T.F. (1991) *Crit. Rev. Oral Biol. Med.* 2, 297-322.

Golub, L.M., Sorsa, T. & Suomanlainen, K. (1992) *Curr. Opin. Dent.* 2, 80-90.

Greenwald, R.A. (1994) *Annals N.Y. Acad. Sci.* 732, 181-198.

Greenwald, R.A. & Golub, L.M., eds. (1994) Inhibition of Matrix Metalloproteinases: Therapeutic Potential. *Annals N.Y. Acad. Sci* Vol. 732, 1-507.

Mitscher, L.A. (1978) *The Chemistry of the Tetracycline Antibiotics*, Ch. 6, Marcel Dekker, New York.

Mohtai, M., Smith, R.L., Schurman, D.J., Taub, Y., Torti, F.M., Hutchinson, N.I., Stetler-Stevenson, W.G. & Goldberg, G.I. (1993) *J. Clin. Inv.* 92, 179-185.

Ramamurthy, N., Greenwald, R., Moak, S., Scuibba, J., Goren, A., Turner, G., Rifkin, B. & Golub, L. (1994) *Annals N.Y. Acad. Sci.* 732, 427-430.

Rifkin, B.R., Vernillo, A.T., Golub, L.M. and Ramamurthy, N.S. (1994) *Annals N.Y. Acad. Sci.* 732, 165-180.

Tilley, B.C., Alarcon, G.S., Heyse, S.P., Trentham, D.E., Neuner, R., Kaplan, D.A., Clegg, D.O., Leisen, J.C.C., Buckley L., Cooper, S.M., Duncan, H., Pillemer, S.R., Tuttleman, M., Fowler, S.E., Minocycline in rheumatoid arthritis. *Ann. Intern. Med.* 122:81-89 (1995).

Uitto, V.J., Firth, J.D., Nip, L. & Golub, L.M. (1994) *Annals N.Y. Acad. Sci.* 732, 140-151.

Yu, L.P., Jr., Smith, G.N., Jr., Brandt, K.D., Myers, S.L., O'Connor, B.L. & Brandt, D.A. (1992) *Arthritis Rheum.* 35, 1150-1159.

CLAIM(S)

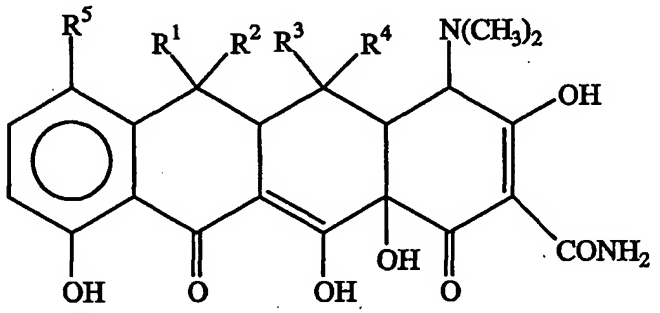
I claim:

1. A method of treating or preventing a disease in a mammal comprising administering an amount of a tetracycline compound sufficient to downregulate microglia expression in the mammal, the disease being Alzheimers' disease, Guillain Barré syndrome, adreneoleukodystrophy, Parkinson's disease, or amyotrophic lateral sclerosis.

2. A method of downregulating microglia expression in a mammal, comprising administering to the mammal in need thereof an effective amount of a tetracycline compound.

3. A method of inhibiting inflammatory activity associated with microglial activation and production, comprising administering to a mammal in need thereof an effective amount of a tetracycline compound.

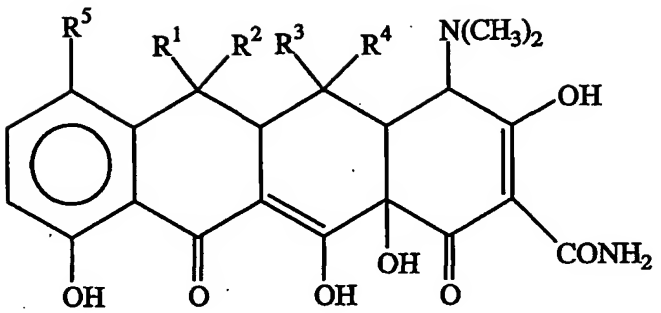
4. The method of claim 1 wherein the tetracycline compound is a compound of formula (I)




(I)

wherein $R^1$ is $CH_3$ or OH and $R^2$ is H or OH, or $R^1$ and $R^2$ taken together are $=CH_2$, $R^3$ and $R^4$ are H or OH and $R^5$ is Cl or $N(CH_3)_2$.

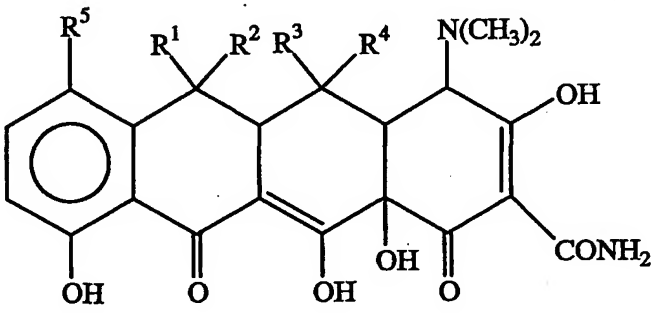
5. The method of claim 2 wherein the tetracycline compound is a compound of formula (I)




(I)

wherein $R^1$ is $CH_3$ or OH and $R^2$ is H or OH, or $R^1$ and $R^2$ taken together are $=CH_2$, $R^3$ and $R^4$ are H or OH and $R^5$ is Cl or $N(CH_3)_2$.

6. The method of claim 3 wherein the tetracycline compound is a compound of formula (I)




(I)

wherein $R^1$ is $CH_3$ or OH and $R^2$ is H or OH, or $R^1$ and $R^2$ taken together are $=CH_2$, $R^3$ and $R^4$ are H or OH and $R^5$ is Cl or $N(CH_3)_2$.

7. The method of claim 1 wherein the tetracycline compound is minocycline or doxycycline.

8. The method of claim 3 wherein the effective amount is an antiinflammatory effective amount.

9. The method of claim 1 wherein the tetracycline compound is a non-antibiotic tetracycline compound.

10. The method of claim 2 wherein the tetracycline is minocycline or doxycycline.

11. The method of claim 2 wherein the tetracycline is a non-antibiotic tetracycline.

12. The method of claim 3 wherein the tertracycline compound is minocycline or doxycycline.

13. The method of claim 3 wherein the tetracycline is a non-antibiotic tetracycline.

14. The method of claim 1 wherein the effective amount is about 0.1 mg/kg/day to about 45 mg/kg/day.

15. The method of claim 10 wherein the effective amount is about 1 mg/kg/day to about 18 mg/kg/day.

16. A method of reducing the neurologic symptoms associated with increased expression of microglia or increased microglia cell production in a mammal, the method comprising administering an effective amount of a tetracycline compound to the mammal.

17. The method of claim 3, wherein an antiinflammatory agent is co-administered with the tetracycline compound.

18. The method of claim 12 wherein the tetracycline compound is administered over a period of time until the mammal becomes asymptomatic or the symptoms are reduced by at least 50% compared to pre-treatment symptoms.

19. A pharmaceutical combination comprising a first antiinflammatory agent which is a tetracycline compound and a second antiinflammatory agent.

20. The method of claim 1 wherein the tetracycline compound is administered parenterally , internally or via a controlled release formulation.

21. The method of claim 1 wherein the amount is below the antibiotic effective amount.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/27593

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/65

US CL : 514/152, 154

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/152, 154

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS on-line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>--<br>Y | Database Medline on STN. Document number 99080089. YRJANHEIKKI et al. "Tetracyclines inhibit Microglial activation and are neuroprotective in global brain ischemia", abstract, Proceedings of the National Academy of Sciences, USA. (22 December 1998) 95(26) 15769-74. | 2, 3, 5, 6, 8, 10-13, 15, 16<br>-----<br>1, 4, 7, 9, 14, 17-21 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 12 NOVEMBER 2001 | 02 JAN 2002 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>PHYLLIS SPIVACK<br>Telephone No. (703) 308-1235 |

Form PCT/ISA/210 (second sheet) (July 1998)★